

# The bioactive peptide endothelin causes multiple biologic responses relevant to myocardial and vascular performance after cardiac surgery

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Over one-half million cardiac surgical procedures are performed in the United States and a large number of these procedures require cardiopulmonary bypass (CPB) and cardioplegic arrest. However, transient left ventricular (LV) dysfunction can occur with reperfusion and separation from CPB and result in a complex postoperative course. The causes for this LV pump dysfunction are multifactorial and include intrinsic defects in myocardial contractile performance and alterations in hemodynamic loading conditions. Neurohormonal system activation and subsequent release of bioactive peptides occur during and after CPB and cardioplegic arrest. One of the more potent bioactive peptides released into the systemic vasculature under these conditions is endothelin-1 (ET).<sup>1-9</sup> Increased synthesis and release of ET has been implicated to exacerbate LV pump dysfunction in a number of cardiovascular diseases.<sup>8-12</sup> Thus, elevated ET in patients during and after cardiac surgery may compromise LV function and contribute to a complex postoperative course.<sup>13-17</sup> In the two companion studies by Verma and colleagues<sup>18,19</sup> in this issue of the *Journal*, of Thoracic and Cardiovascular Surgery, the authors demonstrated that ET can contribute to myocardial cell injury and microvascular constriction after an episode of controlled ischemia-reperfusion, which was particularly pronounced in the context of hyperglycemia or diabetes. These findings add to the body of knowledge to suggest that ET contributes to abnormalities in cardiovascular regulation after cardioplegic arrest and CPB and can further exacerbate these processes in pre-existing disease states such as diabetes. Comprehensive reviews and texts on the molecular biology, biosynthesis, and signaling of ET have been published.<sup>7-10</sup> However, a brief review with respect to the biology of ET and the different cellular functions within the cardiovascular system would be appropriate to place these recent studies into context with our emerging understanding of the importance of ET in the cardiac surgical setting.

## ET and ET Receptor Systems

The mature 21 amino acid peptide endothelin-1, or ET-1, is synthesized from a 38 amino acid precursor, also known as "big endothelin." Big ET is then converted to the biologically active ET-1 by an ET-converting enzyme.<sup>7</sup> The diverse physiologic actions of ET-1 appear to be mediated through two receptor subtypes, the ET<sub>A</sub> and ET<sub>B</sub> receptors. However, the relative affinity of ET-1 for these receptor subtypes is equivalent. In light of the fact that ET-1 appears to be the most potent species of ET and has a high and equal affinity for both major receptor subtypes, then these peptide species are often referred to in generic terms as ET. The production of ET was first described in endothelial cells, but the synthesis of ET has now been identified to occur in a number of cell types including smooth muscle cells and cardiac myocytes.<sup>18,20-22</sup> Thus, the production and release of ET can occur locally within the vascular and myocardial compartments, which in turn will regulate a number of physiologic processes.

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## ET and Vasoconstriction

ET causes potent vasoconstriction of several vascular systems including the myocardial and pulmonary vascular systems.<sup>7-12</sup> Potent constriction of vascular smooth muscle occurs primarily through binding of ET to the ET<sub>A</sub> receptor and, through several intracellular signaling events, increases calcium availability to the contractile elements. The ET<sub>B</sub> receptor contributes to the regulation of vascular smooth muscle tone in several different ways. First, ET<sub>B</sub> receptors located on endothelial cells mediate vasodilation via the release of nitric oxide and prostacyclins.<sup>7-9</sup> Second, this receptor subtype can also exert vasoconstriction when located on the smooth muscle cells.<sup>10,12,23</sup> Therefore, the net contractile effect of ET depends mainly on the relative density of ET<sub>A</sub> and ET<sub>B</sub> receptors on smooth muscle cells and of ET<sub>B</sub> receptors on endothelial cells. It has been reported previously that ET can induce a robust and prolonged vasoconstriction of internal thoracic artery and saphenous vein segments harvested for use as conduits in coronary revascularization procedures.<sup>5,24,25</sup> Moreover, the vasoconstrictive effects of ET are more pronounced in arteries with atherosclerotic disease, and ET amplifies coronary artery contractions induced by norepinephrine and serotonin.<sup>24,25</sup> Coronary atherosclerotic disease and heightened neurohormonal system activation coexist in the cardiac surgical setting and therefore may enhance the vasoconstrictive effects of ET within the myocardial vasculature. Past studies have demonstrated that alterations in ET sensitivity can occur within the systemic vasculature in diabetes.<sup>26,27</sup> In the present report by Verma and colleagues,<sup>19</sup> ET mediated a potent vasoconstrictive effect within the myocardial microvasculature, which was particularly pronounced in diabetic samples after CPB. These investigators reported diminished ET-mediated nitric oxide release in the diabetic myocardial samples. Thus, these new results coupled with past observations suggest that defects in the ET<sub>B</sub> receptor system occur in diabetes, which would result in an exaggerated vasoconstrictive response to ET.

## ET and Cardiac Myocyte Function

Whereas the vasoconstrictive effects of ET are widely recognized, activation of the ET<sub>A</sub> receptor has direct effects on myocyte biology, including contractile protein interactions, inotropic state, protein expression, and electrophysiology. Studies from this laboratory and others have demonstrated abundant myocyte sarcolemmal ET receptors that are primarily of the ET<sub>A</sub> receptor subtype.<sup>21,22,28</sup> Furthermore, these past studies demonstrated that the LV myocyte has the capacity to synthesize and release immunoreactive ET and activate local sarcolemmal ET<sub>A</sub> and ET<sub>B</sub> receptors. Thus, in addition to the systemic effects of ET, local myocyte ET production and ET<sub>A</sub> receptor activation may directly influence LV contractile processes. Recent studies have demonstrated that persistent activation of these ET receptor path-

ways can directly result in a negative contractile effect in isolated LV myocytes, myocardial preparations, and in the intact LV.<sup>21,22,33-36</sup>

Fundamental intracellular events that have been reported to occur after ET<sub>A</sub> receptor activation are the release or release and mobilization of intracellular calcium (Ca<sup>+2</sup>) and intracellular pH changes.<sup>28-32</sup> Since ET<sub>A</sub> receptor activation can cause increased release of intracellular Ca<sup>+2</sup>, then activation of this receptor system after cardioplegic arrest and reperfusion would potentially exacerbate intracellular Ca<sup>+2</sup> homeostasis and contractile function. An important sarcolemmal exchange system that directly influences intracellular pH is the Na<sup>+</sup>/H<sup>+</sup> exchanger. Intracellular pH under normal ambient conditions is maintained relatively alkaline when compared with the extracellular environment, and this is achieved through the transport of protons out of the myocyte. This exchange system has the capacity to correct an intracellular acid load during periods of ischemia through an acceleration of H<sup>+</sup> extrusion and intracellular accumulation of Na<sup>+</sup>. This increased intracellular Na<sup>+</sup> can, in turn, increase the exchange rate of the Na<sup>+</sup>/Ca<sup>+2</sup> exchanger with a subsequent accumulation of intracellular Ca<sup>+2</sup>. Several studies have demonstrated that the Na<sup>+</sup>/Ca<sup>+2</sup> exchanger directly contributes to the increased influx and accumulation of Ca<sup>+2</sup> during ischemia and reperfusion.<sup>37,38</sup> Thus, during early reperfusion, intracellular Ca<sup>+2</sup> homeostasis could be further aggravated by the activation of the Na<sup>+</sup>/H<sup>+</sup> exchanger.<sup>39,40</sup> It has been demonstrated that activation of the ET<sub>A</sub> receptor caused increased activity of the Na<sup>+</sup>/H<sup>+</sup> exchanger, intracellular alkalosis, and altered myocyte contractile performance.<sup>41,42</sup> Thus, enhanced ET<sub>A</sub> receptor activation may further exacerbate the intracellular ionic homeostasis that can occur after cardioplegic arrest and reperfusion. In the companion study by Verma and colleagues,<sup>18</sup> a human myocardial cell system was established and maintained under hyperglycemic conditions as well as after simulated ischemia-reperfusion.<sup>18</sup> Several important findings regarding the ET system were identified from this study. First, a human myocardial cell culture system synthesized ET under basal conditions and was enhanced after ischemia-reperfusion. Second, the elaboration of ET with ischemia-reperfusion activated the ET<sub>A</sub> receptor and subsequently caused cell injury. Third, the ET<sub>A</sub> receptor-mediated cell injury was further pronounced in myocardial cell cultures maintained under hyperglycemic conditions. Thus, an acceleration of ET synthesis within the myocardial compartment with ischemia and reperfusion can cause detrimental effects on myocyte contractile performance and viability.

## Increased ET after Cardioplegic Arrest and CPB

A 3- to 6-fold increase in systemic ET levels has been documented to occur immediately after cardioplegic arrest and reperfusion.<sup>1-6,19</sup> A number of clinical studies have demonstrated that increased ET levels persist well into the

postoperative period.<sup>1-5</sup> The underlying mechanisms for the increased perioperative ET levels observed during and after cardiac surgery are probably multifactorial, including reduced ET clearance during CPB as a result of reduced pulmonary vascular flow, damage to endothelium as a result of absent pulsatile perfusion, increased myocardial ET production and release after reperfusion, increased stress from surgery, and enhanced ET biosynthesis due to thromboxane release from activated platelets. Animal studies have demonstrated a causative relationship between increased ET release and alterations in cardiovascular performance after cardioplegic arrest and CPB.<sup>14-17</sup> Clinical studies have reported that elevated plasma ET levels in the early post-CPB period can be associated with a more complex postoperative course.<sup>4,5</sup> However, ET is synthesized and released into the local interstitial space and, therefore, levels of this peptide that are detected in the systemic circulation likely reflect spill-over from local tissue compartments.<sup>6,18,20-22</sup> With the use of microdialysis techniques, ET levels within the myocardial interstitium can be over 10-fold higher than systemic levels, and a rapid release of ET can occur within the myocardial compartment with cardioplegic arrest and reperfusion.<sup>6</sup>

The physiologic consequences of increased local myocardial ET production in the early post-cardiac surgical setting include vasoconstriction of the native coronary vasculature and newly placed coronary conduits that will hamper blood flow delivery to the vulnerable myocardium. In addition, increased ET exposure of myocytes immediately after cardioplegic arrest can exacerbate contractile function and inotropic responsiveness.<sup>14</sup> Specifically, increased ET release and activation of myocyte ET receptors likely interfere in  $\beta$ -adrenergic-mediated inotropic response in this critical postoperative period. These past observations, as well as the reports by Verma and colleagues,<sup>18,19</sup> suggest that ET may directly contribute to a number of pathophysiologic processes that can occur after cardioplegic arrest and CPB.

### ET Receptor Antagonists and Potential in Cardiac Surgery

Potent and specific ET<sub>A</sub> receptor antagonists, as well as combined ET<sub>A</sub>/ET<sub>B</sub> receptor antagonists, have been described.<sup>8-12</sup> These new nonpeptide ET receptor antagonists are constructs with significant bioavailability, prolonged half-life, and high specificity. ET receptor antagonists have been successfully used in patients with pulmonary hypertension and heart failure.<sup>10-12</sup> These ET receptor antagonists have been successfully used in several animal models of cardioplegic arrest and CPB.<sup>13,15-17</sup> The ET receptor antagonist with the greatest clinical profile to date is the nonselective antagonist bosentan.<sup>12,43,44</sup> Specifically, the immediate administration of bosentan in patients with heart failure

has provided favorable effects on systemic hemodynamics and pulmonary hypertension.<sup>12,44</sup>

A clear change in the patient demographics for cardiac surgery such as coronary revascularization is ongoing, with the patients being older, having pre-existing abnormalities in LV structure and function, and having an increasing incidence of concomitant disease processes such as diabetes. The well-constructed series of in vitro studies performed by the Toronto cardiac surgery group demonstrated that the elaboration of ET after ischemia and reperfusion or after cardioplegic arrest can detrimentally affect a number of cardiovascular processes, particularly in the context of diabetes.<sup>18,19</sup> Moreover, these investigators clearly demonstrated that ET receptor blockade could abrogate the deleterious effects of ET. An intravenous formulation of an ET antagonist has been developed and acute infusions of this mixed ET antagonist have been safely deployed in patients with severe heart failure.<sup>44</sup> Although the effects of ET on myocardial function have received significant attention in heart failure, the potential deleterious effects of increased ET release and/or production within the myocardium after cardioplegic arrest and reperfusion and the underlying mechanisms for these effects are beginning to emerge. The time for well-designed, hypothesis-driven, preclinical and clinical studies regarding the mechanistic role of ET to contribute to postoperative recovery with respect to LV function, systemic hemodynamics, and coronary graft viability is now upon us.

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